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TECHNOLOGICAL IMPROVEMENTS FOR THE CULTIVATION OF INVERTEBRATES AS FOOD FOR FISHES AND CRUSTACEANS. II. HATCHING AND CULTURING OF THE BRINE SHRIMP, *ARTEMIA SALINA* L.

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ABSTRACT

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Although extensive fundamental research has been performed on the brine shrimp, the yields from hatching and culturing this organism which is widely used in mariculture, can be greatly improved. By exposing the immersed cysts to light and by using a new type of hatching cylinder and separator box, greater hatching and separation efficiencies can be obtained. Controlled mass culture of brine shrimp larvae to the adult stage has been extremely difficult until now; the literature on the subject is reviewed and a new technique of high density culturing at the pilot-stage is described.

INTRODUCTION

"Expanding the rearing techniques presently used to mass culture systems may be difficult if a suitable food is not available in sufficient quantity" (Houde, 1973).

In part I of this paper (Persoone and Sorgeloos, 1975) we emphasized the necessity of finding "inert" alternatives for live food in order to reduce the number of living links and the fundamental and/or technical difficulties which they involve. At present, unfortunately, we are far from this goal and have to rely on small living organisms, cultured in high densities, for feed. Reviewing the literature, it is striking how small the number of available, suitable live food species is. In fact the only organism on which about 99% of culturists have relied, is the brine shrimp *Artemia salina*, widely used as food for fish larvae (Seale, 1933; Rollefsen, 1939; May, 1970, 1971), decapods (Williamson, 1968) and cephalopods (Von Boletsky, 1975). All over the world, experiments on the mass culture of other organisms are in progress; in Japan the results obtained with the rotifer *Brachionus plicatilis* look most promising (Mock, 1971; Hirayama, personal communication.

The principal reason why brine shrimp larvae are so widely used for mariculture purposes is undoubtedly that their culture can be started from an (apparently) inert source; namely dried eggs which need only be hatched. Although extensive fundamental research has been performed on this particular species (see reviews of Littlepage and McGinley, 1965; Nimura, 1967; Ivleva, 1969), there are still a number of practical problems with hatching, and some totally erroneous principles are, unfortunately, deeply entrenched. Even though *Artemia* larvae of increasing sizes (thus of different ages) would be extremely welcome for feeding larger larval fishes or crustaceans, their controlled mass culture has almost completely failed until now.

HATCHING OF THE CYSTS

An extensive literature exists on the hatching of *Artemia* eggs (in reality cysts, since embryological development has already occurred, up to the gastrula stage (Fautrez-Firlefijn and Van Dijck, 1961)) and on the mechanisms involved in the restarting of the "biological clock" in these cysts. At least four essential prerequisites are necessary to restart the embryological development, leading to the hatching of the nauplii:

(1) *Hydration of the cysts in sea water.* Salinity and osmotic tolerance ranges have been reported by Boone and Baas Becking (1931), Jacobi and Baas Becking (1933), Baas Becking et al. (1936), Jennings and Whitaker (1941), Clegg (1964) and Von Hentig (1971). The best hatching conditions are obtained in salinities ranging from 5 to 70‰.

(2) *Oxygenation of the medium.* Gilchrist (1954) reported that 3 ppm was the minimum oxygen concentration in which *Artemia* cysts could hatch. Recent studies of Nimura (1968) corroborated by some of our own experiments (Sorgeloos, unpublished data) reveal that the hatching rate of *Artemia* cysts from the U.S.A. (Californian strain) is constant in the range of 2 to 8 ppm dissolved oxygen. Below this value, the hatching efficiency decreases and it is completely inhibited at 0.6 to 0.8 ppm.

Continuous aeration, which keeps the cysts in suspension, is beneficial to hatching (Dutrieu, 1960 a; Teramoto and Kinoshita, 1961). When cysts accumulate at the bottom, many become rapidly subjected to anaerobic conditions, and this results in the embryological development being blocked (Dutrieu and Cresta-Blanchine, 1966).

(3) *Illumination of the hydrated cysts.* Hatching experiments with cysts from Bulgaria and the U.S.A. (Utah and California) revealed that light triggers the internal "biological clock" to start again. The hatching efficiency was indeed considerably higher in light compared to dark (respectively 26, 48 and 62% in dark, versus 40, 73 and 95% in light conditions) (Sorgeloos, 1973 b). Other experiments (Sorgeloos, unpublished data) showed that brief illumination of the cysts after hydration is sufficient to ensure a good hatching rate. The minimum exposure time depends on the strain used and on the light intensity (the critical wavelength has not yet been determined). For Californian cysts, illumination for 10 min at an intensity of 2 000 lux is sufficient.

The light triggering is only effective in aerobic conditions. The practical consequence of this discovery is that the technique generally applied, hatching *Artemia* eggs in darkness, is completely erroneous, since many cysts will not have received a sufficient quantity of light at immersion, to induce the photo-oxidative trigger.

(4) *Temperature.* Boone and Baas Becking (1931) reported that the optimum hatching temperature was approximately 30°C. Carrying out a detailed study on the influence of temperature on the hatching process, Von Hentig (1971) found that the maximum efficiency is obtained between 20°C and 30°C; the hatching rate, however, is fastest at 30°C (50% hatching after 2 days at 30°C versus 3.5 days at 20°C). Our experiments with different *Artemia* strains indicated that the optimum hatching temperature varies from race to race: e.g., more than 50% hatching after 36 h for California—U.S.A. at 28°C; Utah—U.S.A. at 30°C; People's Republic of China at 35°C (Sorgeloos, unpublished data; confirmed by Takano, personal communication).

Thus, to obtain good hatching, the eggs must be exposed to light (at least just after hydration) in order to insure the embryo's development is triggered; the medium must be continuously oxygenated, and the eggs kept in suspension.

Two systems for hatching brine shrimp eggs are commonly used in hatcheries:

(1) Rectangular hatching and separator boxes, originally described by Keliherer (1952), improved by Shelbourne et al. (1963) and Riley (1966) and further automated by Shelbourne (in Costlow, 1969) and Nash (1973). Due to its flat bottom with a large area, strong aeration is needed to maintain circulation of the eggs in the vessel. Shelbourne (in Costlow, 1969) admitted that ". . . a flat-bottomed container doesn't work well because *Artemia* get driven into the corner and hatching rates are then invariably low". Nash (1973) further explained that there is also a ". . . loss of some viable eggs marooned on the sides of the internal surfaces owing to the strong aeration employed". The absence of any illumination during the immersion of the cysts must certainly affect the hatching efficiency. Only small densities, ranging from 0.3 to 1.0 g cysts per l are commonly hatched in these systems.

(2) Large opaque plastic water vats as described by Jones (1972). Both the absence of light (opaque containers) and the aeration being too vigorous (necessary to avoid sedimentation on the flat bottom) decrease the hatching efficiency to a level far below that which can be achieved when the prerequisites of light, oxygen and circulation are provided. The density of eggs used in this system is only 0.3 g cysts per l.

We finally found a practical solution to these problems by using glass or transparent plastic cylinders, funnel-shaped at the base, aerated from the bottom (Fig.1 in Persoone and Sorgeloos, 1975). Freshly hatched *Artemia* larvae are quite sensitive to vigorous air-bubbling, but regular, slight aeration (1 l air per min) suffices to aerate the medium and to keep all the cysts in suspension. To prevent foaming, a few drops of a non-toxic silicone antifoamer is added. In our cylindrical tubes we regularly hatch densities of 10 g cysts per l with a yield of 70% with Utah cysts and 90% with the Californian strain.

SEPARATION OF THE HATCHED NAUPLII FROM THE HATCHING DEBRIS

It is of paramount importance that after hatching, *Artemia* larvae be separated from the unhatched or empty cysts. Indeed, Morris (1956) and Rosenthal (1969) showed that fish larvae starve if they eat empty brine shrimp shells. Herald and Rackowicz (1951) even reported a "blockage of the gut".

The positive phototactic behaviour of the nauplii can be exploited for this separation (Anonymous, 1948). Directing a light beam on the (transparent) hatching device (mostly glass vessels) results in the larvae swimming towards the light. They can then be siphoned off at that particular place. This rough separation technique, although commonly used (Keliherer, 1952; Galen, 1969; Jones, 1972), has various disadvantages:

(1) It is time consuming (according to Jones (1972) the separation time should be 75 min) and it takes skill to remove the larvae without siphoning off the debris accumulated on the bottom and the surface.

(2) Separation is qualitatively and quantitatively far from being optimal. Many empty egg shells have exactly the same density as the medium and consequently will be siphoned off with the larvae.

(3) As the medium is not aerated during the long separation time, this system does not permit the handling of high larval densities without the risk of the nauplii suffering an oxygen shortage (Jones, 1972).

In the *Artemia* separator boxes described by Keliherer (1952), Shelbourne et al. (1963) and Riley (1966), the nauplii, hatched in darkness, swim through holes or slits from the dark compartment to the brighter side. Once separation is achieved, the partition can be closed and the larvae siphoned off. In larger versions of this incubator, the larvae which concentrate in the illuminated area of drain off are separated a second time from the hatching debris that has been carried along into the concentrator, again by light attraction (Shelbourne, in Costlow, 1969; Nash, 1973).

In carrying out experiments with these rectangular separator boxes, we found that the separation was rather poor due to the tendency of the larvae to gather in corners; furthermore, a large number of larvae were too far from the illuminated area to receive the light stimulus and consequently did not move in the desired direction.

To solve these problems, we constructed a cylindrical separator box, which appeared to be very satisfactory since it separated more than 90% of the larvae from the debris (Persoone and Sorgeloos, 1972). Recently, we were able to improve this lab-scale device and to construct a new enlarged version, meeting the needs of large-scale uses. The dimensions of the device should be chosen according to the quantity of nauplii needed. The device can be made of non-transparent PVC in three parts (Fig.1):

(1) A cylinder (2) with several horizontal slits at opposite sides, is glued to the bottom of a larger cylindrical box (1) provided with a tap (5). The slits in

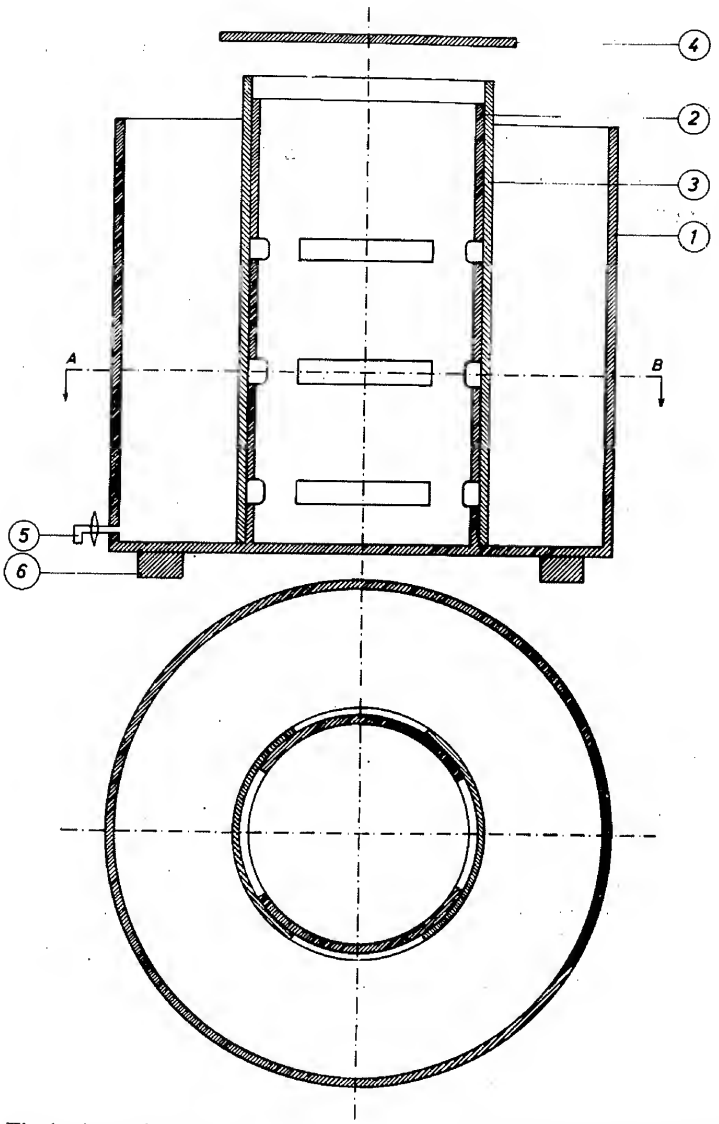

Fig.1. *Artemia* separator box, lateral and A—B section view: (1) cylindrical non-transparent box; (2) and (3) internal separation cylinders provided with slits; (4) lid; (5) drain stop-cock; (6) supports.

cylinder 2 are about 1 cm in width and have a length slightly smaller than the 1/4 of the circumference of the cylinder.

(2) A second cylinder (3) fits tightly over the internal cylinder of the box, and is provided with exactly the same kind of slits.

(3) A flat piece of PVC serves as a cover for the internal cylinder (4).

The separator box is used as follows: after hatching, the mixed suspension

of cysts and nauplii, highly contaminated by bacteria, is first washed over a plankton gauze and diluted with fresh sea water. The larval suspension is then poured into the internal cylinder (closed off from the outer compartment) and sea water is added to a predetermined depth. The outer chamber is then filled with sea water to exactly the same level as the inner compartment. It is very important that the external and internal levels are exactly the same. After placing the cover on top of the internal compartment, a light source is placed above the hatching device and cylinder 3 is twisted 90° to connect the two chambers through the slits. The nauplii in the darkened compartment will immediately start swimming to the light source through the slits. After 10 to 15 min, cylinder 3 is turned in the opposite direction to separate the nauplii from the empty or unhatched cysts. Experience has shown that the separation is excellent, ranging from 90 to 95%, a result which we could never obtain with any other separation technique.

In Table I, the input and output efficiencies (total weight or number of cysts at the start versus total number of nauplii recovered) of our separator box are compared to those of Riley (1966) and Jones (1972). The system of Riley (1966) is an application of the hatching and separator box of Shelbourne

TABLE I

Comparative data on the hatching input and output efficiencies of the presently used large-scale hatching devices

	Riley (1966)	Jones (1972)	Sorgeloos and Persoone (1975)
Hatching system	Opaque rectangular box (92 × 45 cm)	Opaque plastic water vat	Funnel-shaped glass vessel
Volume of the hatching device (l)	50	180	0.5
Origin of the cysts	Utah (U.S.A.)	California (U.S.A.)	Utah (U.S.A.)
Input of cysts (g/l)	±0.3 [1]	±0.6[1]	10
Total hatch (number of nauplii)	1×10^6	8×10^6	0.7×10^6
Density of nauplii (individuals per l)	0.02×10^6 0.0001×10^6 [2]	0.04×10^6	1.4×10^6
Output efficiency			
Number of nauplii per g cysts [3]	70 000 300 [2]	73 000	143 000
Larvae (%) [4]	32.8 0.2 [2]	24.3	65.0

[1] These figures were calculated on a basis of 0.60 g cysts in 1 ml (own results from countings made with Utah and California cysts).

[2] According to Jones, only about 100 nauplii per l could be obtained when using the Riley system.

[3] This figure was calculated on a basis of 220 000 Utah cysts in 1 g or 300 000 California cysts in 1 g (own results).

[4] Optimal hatching efficiency for Utah cysts: 70 to 75% (Von Hentig, 1971; Sorgeloos, 1973); for California cysts: 90% (Sorgeloos, 1973).

et al. (1963), so we shall only comment on the former since exact data on the latter are lacking. Since Shelbourne (in Costlow, 1969) and Nash (1973) fail to give detailed data, we were not able to take their results into consideration.

From the data given in Table I it is clear that, when output efficiency is considered, our system gives at least twice as good results as the commonly used devices. As the hatching efficiency (which depends on the brand used, i.e., geographic race and sorting quality) for this particular Utah stock, amounts to 70—75% and as we obtain a yield of 65%, this means that somewhere in the process we loose at least 5%. This is partly due to mortality, partly to incomplete recuperation.

The practical consequence of this excellent yield is that a considerable number of *Artemia* dry cysts, in comparison to previous techniques, are saved. In our small hatching tubes of 500 ml, 700 000 live nauplii are obtained and this approximates the total output mentioned by Riley (1960), but questioned by Jones (1972), in his 50 l system. (Jones (1972) when using the Riley system only obtained a density of 100 nauplii per l, instead of 20 000 per l as mentioned by Riley.) This means that with six small 1-l tubes we get the same yield as Jones in a vat of 180 l. The practical result is an enormous space and time saving in those aquaculture farms, where *Artemia* nauplii are used. Another advantage is that the usual equipment necessary to hatch *Artemia* eggs (pumps, compressors, hatching boxes or butts) becomes superfluous or can at least be greatly reduced.

CULTURING THE LARVAE

General considerations

In most cases where brine shrimps are used in mariculture farms as live food, the larvae are fed to the desired end product as freshly hatched nauplii. Walne (1967) prudently suggested that ". . . it is possible that as the predator grows, it is desirable for the size of the prey to be increased." Moreover, it appears that the food value of *Artemia* can be changed drastically by feeding it an appropriate food for a short time. Wickins (1972) for example, showed that freshly hatched Utah nauplii, which are unsuited as food for the larvae of the prawn *Palaemon serratus*, became an excellent diet when fed with algae for 24 h.

A most interesting fact is the enormous increase in length and weight of *Artemia* nauplii during growth: freshly hatched nauplii measure approximately 0.4 mm and weigh about 0.002 mg; the adults are about 8 mm long and weigh about 1 mg; a 20-fold increase in dimensions and a 500-fold increase in biomass (Gilchrist, 1956; Mason, 1963; Reeve, 1963 c; Von Hentig, 1971). As the amount of fat in the larvae decreases from 23.2% of the dry weight in the nauplius (0.6 mm length, Dutrieu, 1960 a) to 16.5% in the metanauplius (2.5 mm, Dutrieu, 1960 a) and to 7.0% in the preadult stage (Brick, in Helfrich, 1973), the digestibility of the larvae increases with age. The protein

content also increases rapidly during growth: from 42.5% in the newborn nauplius to 62.78% in the adult stage (Dagg and Littlepage, 1972; Brick, in Helfrich, 1973).

Mass culturing techniques for brine shrimp have not yet been satisfactorily developed, if we believe the scarce literature on the subject. This is more striking because an enormous number of papers have been published on the different aspects of brine shrimp culturing: development of artificial media (Provasoli and Shiraishi, 1959; Provasoli and D'Agostino, 1969); the influence of salinity on *Artemia* rearing (Eliassen, 1952; Gilchrist, 1956; Baid, 1963; Nimura, 1963; D'Agostino and Provasoli, 1968); optimum temperature, salinity and filter-feeding conditions (Reeve, 1963 a, b and c); the use of inert foods (Fautrez-Firlefijn and Van Dijck, 1961; Teramoto and Kinoshita, 1961; Bowen, 1962; Anonymous, 1964); the food value of different unicellular algae (Bond, 1933; Gibor, 1956; Provasoli et al., 1959; Walne, 1967); the influence of various feeding regimes (Mason, 1963; Reeve, 1963 a; Nimura, 1963, 1967; Sushchenya, 1964); oxygen consumption related to growth (Kuenen, 1939; Gilchrist, 1958; Dutrieu, 1960 a, b); energy metabolism (Eliassen, 1952; Gibor, 1957; Sushchenya, 1962, 1964; Reeve, 1963 c; Paffenhöfer, 1967; Khemeleva, 1968) and geographic differences (Kuenen, 1939; Gilchrist, 1960; D'Agostino and Provasoli, 1968).

The best growth data have been obtained by Teramoto and Kinoshita (1961) (mating pairs after 6 to 7 days) and by Walne (1967) (adult stage after 9 days culturing, with 10 animals in 1-l vessels); the highest density in small-scale experiments has been reported by Teramoto and Kinoshita (1961): 1 individual per 2 ml medium (beakers of 500 ml). The described culturing methods are, however, not suited for mass production.

Continuous oxygenation of the medium is deleterious to the larvae (Dutrieu, 1960 a; Nimura, 1967) and even reduces the growth rate (Bond, 1933; Von Hentig, 1971). This is one of the major reasons for failure in the transition from lab-scale to mass culture.

Last but not least, the use of live algae as food for the nauplii is too expensive for application to mass culturing. In the U.S.S.R., *Artemia* is mass cultured (very primitively) in ponds that have been fertilized to enhance bacterial and algal development (Ivleva, 1969). Maximum densities of 400 individuals per l are reached. As the environmental conditions are not constant (temperature, light, rainfall, predation, etc.) culturing conditions and growth rates fluctuate greatly, with the result that standardization is difficult.

In Japan, at the Nagasaki-ken Fisheries Experimental Station (Anonymous, 1964; Nimura, personal communication) *Artemia* larvae are mass cultured in densities of 6 nauplii per l on a diet of mixed soybean powder and flour. Not only is the growth rate quite poor (1.5 to 6.5 mm in 13 days) but Nimura (1967) reported that "when an artificial food is used in breeding, the mortality rate is very high in the larval stages". Mock (1971) reported on *Artemia* culturing at the School of Fisheries, Tokyo University where brine shrimp larvae were fed with the freshwater algae *Chlorella*; further details are lacking.

Personal work

We started tackling the fundamental problems of culturing *Artemia salina* at high densities 5 years ago. The monocellular phytoflagellate *Dunaliella viridis* appeared to be a most appreciated food and it is easy to mass culture in a very simple medium (for details on algal culturing see Persoone and Sorgeloos, 1975). An automatic larval counter was developed to enable easy and fast counting of the nauplii in experiments necessitating many replications and set numbers of larvae (Van Outryve and Sorgeloos, 1975). Several culturing devices were designed for comparative growth experiments (Persoone and Sorgeloos, 1975).

Most of the preliminary research on "optimal culturing conditions" for brine shrimps was carried out in glass culturing tubes ranging from 100 ml to 1 l and contained 1 to 3 nauplii per ml in artificial sea water (formula of Lyman and Fleming, 1940, or Dietrich and Kalle, 1963) at a temperature of 28°C. Semi-continuous or continuous feeding proved to be better than feeding twice a day. Regular renewal of the sea water (at least every other day) was of paramount importance in these high density culturing experiments in which the brine shrimps reached the adult stage within 2 weeks on a diet of live algae.

Comparative experiments on the suitability of various algal species, are very difficult to carry out accurately, for they necessitate continuous availability of algal cultures in the exponential growth phase. We therefore froze algae harvested during the log phase and ran a series of experiments on the influence of the preparation of the food (frozen or dried, versus live algae) on its nutritional value. Although slightly better results were obtained with living algae, it appeared that the preparation of the food did not drastically influence the nutritional value of *Dunaliella viridis* (Sorgeloos, 1975). The important conclusion of these experiments was that ". . . for feeding purposes algae can be mass cultured, harvested during their exponential growth phase and stored at will for later use" (Sorgeloos, 1975) and this solves many practical problems encountered by various researchers at the moment.

From experiments with commercially available dried freshwater algae (Clement and Van Landeghem, 1970; Soeder et al., 1971; Feldheim, 1973) it appeared that heated-drum dried *Scenedesmus*, as well as freeze-dried *Spirulina* are suitable food for brine shrimp. This finding led us to reinvestigate high density culturing of *Artemia* on an economic basis. The use of inert food particles led us to discover an interesting phenomenon, namely the influence of light on the growth rate of *Artemia* larvae. Larvae fed with exactly the same quantities of dried algae grew significantly faster in darkness than in light (Sorgeloos, 1972). This is probably due to the much greater swimming activity (often resulting in crowding) of the positive phototactic larvae when cultured in the light. On an energetic basis, larvae cultured in darkness spend less energy swimming and can thus grow more rapidly.

For the small-scale culture of *Artemia*, we use funnel-shaped vessels (100

ml to 1 l) (Persoone and Sorgeloos, 1975). Short slow bursts of air bubbles (only 5 sec long, four times an hour) from the bottom of these tubes, assures resuspension of the food particles, an even redistribution of the larvae and sufficient oxygenation of the medium.

From these lab-scale (though high density) culturing columns, we turned to pilot-scale, non-automatic 30-l culturing columns, made of plastic sheet (Sorgeloos, 1973 a). These are the same cylinders as are regularly used in our laboratory for mass culturing of algae (Persoone and Sorgeloos, 1975). With two feeds a day, and renewal of the water every other day, we succeeded in growing 100 000 larvae to the adult stage in 12 days, with a mortality of less than 10%. Here too, a periodic emission of air bubbles from the bottom of the column is used.

Recently, we made the system automatic with a time-switch, electromagnetic valves and a multi-channel pump (see Fig.2), which theoretically enables us to run an indefinite number of 30-l columns. This automatic *Artemia* culturing set works as follows: Dried *Scenedesmus* flakes are disintegrated in a simplified and enlarged version of the pearl mill described by Novotny (1964) and Zahradnik (1967). The algal suspension is diluted to a concentration of 10 to 15 g per l and protected against bacterial decomposition by addition of 200 g NaCl per l. It is then distributed among the 1-l algal tubes with funnel-shaped bottoms, from which it can be transferred to the cultures through tubes connected to a multi-channel pump. The algae can either be distributed to all the columns from one storage cylinder, or preferably from separate cylinders (as shown in Fig.2) permitting the quantity of food to be adjusted for each culture. The latter set-up allows the system to run continuously and a daily harvest of one cylinder, i.e., approximately 60 000 to 90 000 adult *Artemia.*

Each hour the programmer (a cam timer with four discs) triggers the magnetic valve for the compressed air (MV 1). A 30-sec stream of air bubbles resuspends the food in the algal tubes. The addition of a few drops of a non-toxic silicon anti-foamer (harmless to *Artemia,* even after ingestion) prevents possible foaming. About 20 sec later, the programmer starts the multi-channel pump which distributes food to the different columns for a specified time.

In order to avoid clogging and fouling of the feeding tubes, we developed an automatic rinsing system, which, at the end of the feeding period, cleans the tubes by passing water followed by compressed air through valve 4. Once rinsing and drying is completed, the programmer commands a short flow of air bubbles, which each 15 min aerates the cultures and resuspends the food and animals throughout the whole column.

Data obtained with this system are quite promising: only 10% mortality with densities of 2 to 3 larvae per ml (60 000 to 90 000 per column) which reach the adult stage within 10 days (the larvae measure about 2 mm within 3 days and 5 to 6 mm after 8 days).

We are on the verge of mass-scale culturing in 500-l containers, automating

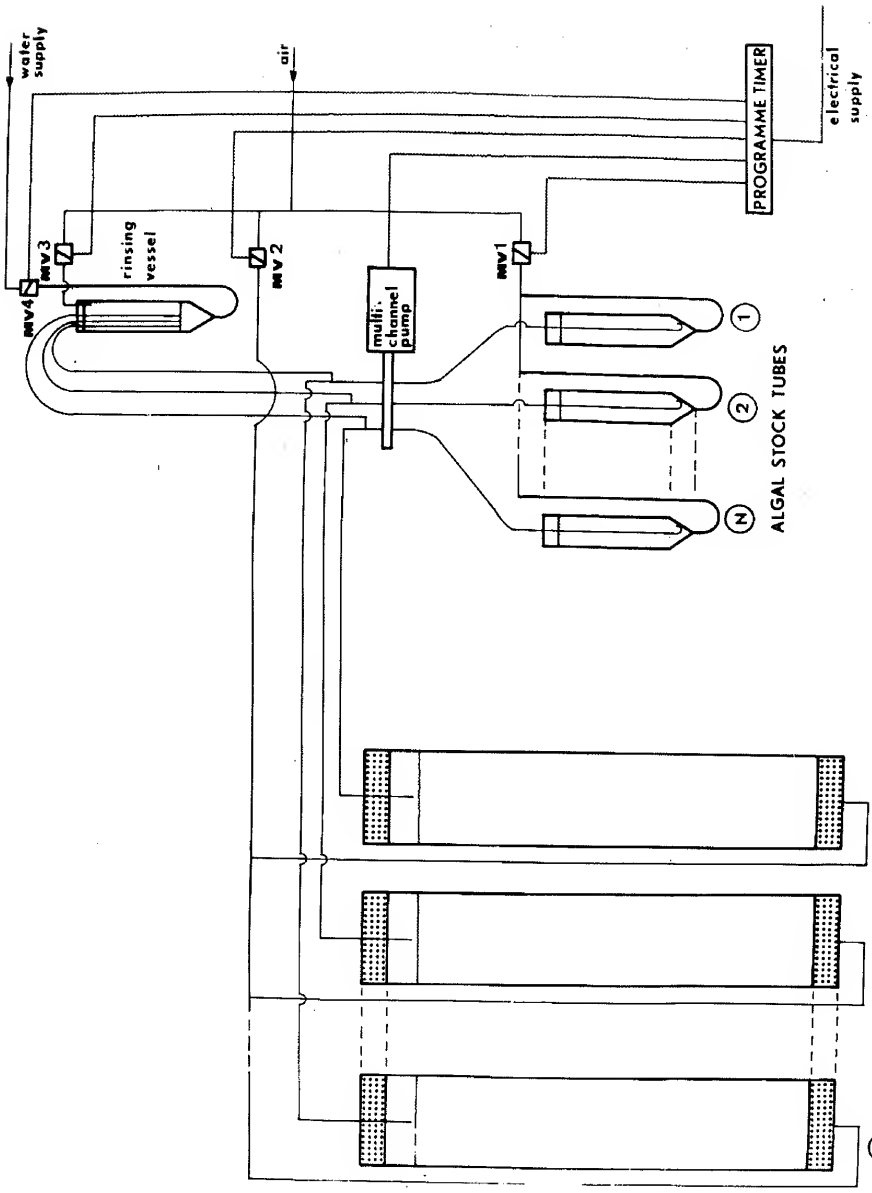

Fig. 2. Automatic culturing set for high density culturing of *Artemia* larvae.

the system further with a daily automatic renewal of the medium. When we extrapolate our results from the 30-l columns to these new 500-l containers, and assume a 50% success rate, this would mean that within 10 days we could harvest 750 000 adult brine shrimps from each container. Using the conversion factors of Reeve (1963 c) and Gun'ko (in Ivleva, 1969), this represents approximately 0.5 kg dry weight, or about 3.5 kg wet weight. If we compare our actual 30-l results, i.e., 208 g/m^3/day and our calculations for the 500-l containers, i.e., 100 g/m^3/day, with the production data of Nechaev (in Ivleva, 1969) in outdoor culturing ponds in the U.S.S.R., namely 5 g/m^3/day, we are able to produce with our indoor culturing systems 20 to 40 times as many adults!

Although the recent drafts of *Artemia* aquaculture proposals (Bowen, 1968; Webber, 1969; Helfrich, 1973) only consider outdoor culturing, we think that with the data mentioned, indoor farming should certainly be considered, especially in view of the fact that the system is automatic and there is continuous control of the culturing conditions. Most of the culturing problems seem to be solved at the present time, so it is now up to the economist to take the non-biological aspects into consideration.

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